Determining the Optimum Night Length for Flower Development in a Modern Poinsettia Cultivar

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Abstract. The effect of night length (NL) on the flower development of poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) ‘Prestige Red’ was evaluated. Flower initiation occurred by subjecting plants to a 14-hour NL for 10 or 17 days, termed short-day (SD) treatments, and then transferring the plants to each of four NL treatments (11, 12, 13, or 14 hours) to observe the effects of NL on flower development. The plants grown continuously with the 14-h NL treatment were the control group. The timing of first color, visible bud, and anthesis were recorded during flower development, and bract and leaf data were collected at anthesis. Leaf number was unaffected by the SD or NL treatments, suggesting that flower initiation occurred during the 10-day SD treatment before the start of NL treatments; thus, the NL treatments only affected flower development. The timing of first color and visible bud were significantly delayed with the 10-day SD × 11-hour NL treatment relative to the 14-hour NL control; however, first color and visible bud were not delayed with the 17-day SD × 11-hour NL treatment. The 11-hour NL treatment resulted in fewer plants reaching anthesis, and these plants had fewer stem bracts and less bract color development compared with the 12-hour, 13-hour, and 14-hour NL treatments. Therefore, an 11-hour NL is suboptimal for flower development; nonetheless, significant development did occur. The 12-hour NL resulted in less color development than the 13-hour and 14-hour NL treatments in the lowest stem bract positions, but the plants had a commercially acceptable appearance. These results demonstrate that minimal differences in flower development occur with NL ≥12 hours, but that optimal development required NL ≥13 hours.

Poinsettia is an SD plant that forms a determinate inflorescence under inductive photoperiods (Ecke et al., 2004). The inflorescences described in this manuscript are defined as follows: the primary cyathium ends the apical stem and is the first cyathium to reach anthesis; the secondary cyathia consist of three cyathia that subtend the primary cyathium; and one primary bract subtends each of the three secondary cyathia and displays a whorl of three bracts. Stem bracts refer to bracts that develop on the stem below the whorl of primary bracts. Primary and stem bracts display a range in the area of the

bract containing red pigmentation, and the area of pigmentation typically increases with the proximity of the bract to the shoot apex.

Several of the foundational studies on poinsettia flowering used anatomical observations by dissecting apical meristems from plants under inductive photoperiods (Goddard, 1960; Larson and Langhans 1962a, 1962b; Miller and Kiplinger, 1962). In each of these studies, the vegetative meristem was shown to persist for several days after the start of SD until a morphological transformation occurred that physically distinguished the meristem from one that was vegetative. The measurements and descriptions slightly vary among these studies; however, in general, the meristem becomes reduced in height, with a horizontally flat surface, followed by the differentiation of the primordia of the three primary bracts and the primary cyathium. The time from the start of an inductive photoperiod until the transition of the meristem from vegetative to reproductive development is defined as flower initiation, i.e., the sum of the various physiological and biochemical responses to inductive photoperiods that elicit a morphological change at the shoot apex. Flower development is defined as the sum of all floral

development events downstream of the first observable change at the apex.

To evaluate the effects of NL on flower initiation, several studies have subjected poinsettias to various photoperiods at moderate temperatures and dissected shoot tips to determine when flower initiation has occurred (Larson and Langhans, 1962b; Miller and Kiplinger, 1962; Wang, 2001; Wieland, 1998). For example, Larson and Langhans (1962b) reported that flower initiation of ‘Barbara Ecke Supreme’ occurred after 14, 16, 18, and 30 d when subjected to NLs of 16, 15, 14, and 12 h, respectively. Miller and Kiplinger (1962) found similar results for ‘Barbara Ecke Supreme’: flower initiation occurred at 14, 16, 27, and 65 d when subjected to NLs of 14, 13, 12, and 11 h. Grueber and Wilkins (1994) subjected poinsettia cultivars ‘Brilliant’ and ‘V-14’ to 16-h NLs or natural day photoperiods starting on 3 Sept. (lat. 44°57’N) and reported that flower initiation occurred within 6 to 10 d under the 16-h NL treatment, whereas flower initiation occurred within 19 to 24 d under the natural day photoperiods. These results demonstrate that flower initiation occurs more rapidly at NLs between 13 and 16 h than at NLs of 11 to 12 h; therefore, when black cloth is used to artificially extend the NL to 13 h or more, poinsettias will initiate flowers faster than when provided natural NLs during the fall.

The photoperiod requirements for flower initiation and flower development can be different for the same plant. For example, chrysanthemums (*Dendranthemum ×grandiflorum*) are SD plants that will initiate flowers under photoperiods that are insufficiently short for flower development (Cockshull, 1976). Similarly, poinsettias maintained under long-day conditions will initiate a flower bud when a cultivar-specific node number has been achieved, but the bud will fail to develop into a functional flower under those same long-day conditions (Evans et al., 1992). In both species, flower initiation may occur with photoperiods under which flower development otherwise fails. Furthermore, different phases of flower development of poinsettia have varying degrees of sensitivity to the photoperiod. For example, Miller and Kiplinger (1962) reported that the time from flower initiation to a macroscopically visible flower bud, termed visible bud, increased from 17 to 23 to 38 d as the NL decreased from 13 to 12 to 11 h, respectively, for poinsettia ‘Barbara Ecke Supreme’. In contrast, no differences were observed when plants were placed under 15-h NL until visible bud, followed by a shift to NLs of 11, 12, and 13 h until anthesis. These results suggested that the early phase of flower development was sensitive to NL from 11 to 13 h, whereas the development of the inflorescence from visible bud to anthesis was not. Thus, flower initiation and flower development may respond differently to a given NL, and different stages of flower development may also respond differently to NL.

Previous research studying the effects of NL on flower development resulted in conflicting outcomes. For example, Grueber and

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Wilkins (1994) reported that poinsettias forced to flower under 16-h NLs developed faster than poinsettias under natural NLs (lat. 44°57'N) beginning on 3 Sept. from flower initiation to visible bud; however, the inverse was true for flower development from visible bud to anthesis. Miller and Kiplinger (1962) found that NLs more than 13 h decreased the number of days from flower initiation to visible bud; however, no differences were observed among NL treatments (11, 12, 13, 14, and 15 h) from visible bud to anthesis. Wieland (1998) found no differences in the number of days from flower initiation to visible bud or anthesis with a NL of 15 h and natural NLs beginning on 17 Sept. or 6 Oct. (lat. 29°40'N).

Previous studies of poinsettia flower development have not evaluated bract color development beyond recording the date of first color. Bract color development is the primary marketable trait of poinsettia; therefore, understanding how the photoperiod affects color development has significant value for commercial growers, and it cannot be assumed that bract and cyathia development respond similarly to NL.

The objective of this research was to evaluate the effect of NL on the bract color and flower development of a modern poinsettia cultivar and, specifically, to determine whether naturally occurring NLs in October and November are optimal for flower development (i.e., 12-h to 13-h NL compared with a constant 14-h NL).

Materials and Methods

General procedures. Unrooted cuttings of 'Prestige Red' were received from a commercial supplier (Dümmen-Orange, Encinitas, CA) and propagated in a foam medium (Oasis Rootcubes Plus Wedge; Smithers-Oasis, Kent, OH) under LD conditions that consisted of light-emitting diode (LED) bulbs (9-W LED A19 Light Bulb; Utilitech, West Lawn, PA) delivering $1.2 \pm 0.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 1630 HR to 0000 HR daily. After 27 d, rooted cuttings were transplanted to 1.33-L containers with a peat-based growing medium (Fafard 3B; Sun Gro, Anderson, SC). Metal halide lamps provided $40 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 0800 HR to 1730 HR daily, and as night-interruption lighting from 2200 HR to 0200 HR nightly until the start of the SD treatments. Therefore, the lamps provided a daily light integral (DLI) of $2.2 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ during the vegetative phase and $1.6 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ during the flowering phase. The total DLI provided from natural sunlight and the metal halide lamps during the October and January replications were 12.9 ± 6.0 and $12.2 \pm 7.9 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, respectively. Plants were fertilized as needed with Peters Excel Cal-Mag Special ($15\text{N}-5\text{P}_2\text{O}_5-15\text{K}_2\text{O}$) at $150 \text{ mg}\cdot\text{L}^{-1}$ N for the duration of the experiment. Day and night temperatures measured by the greenhouse weather station were $20.9 \pm 1.7^\circ\text{C}$ and $18.3 \pm 1.6^\circ\text{C}$, respectively, for the October replication and $21.4 \pm 2.4^\circ\text{C}$ and $18.3 \pm 1.7^\circ\text{C}$ for the January replication.

Experimental treatments. Uniform poinsettia 'Prestige Red' plants were placed on greenhouse benches to apply the initial SD treatments, which consisted of a 14-h NL provided for 10 or 17 d. These two SD treatments were provided because the exact time required for flower initiation to occur is not known; therefore, the two SD treatments ensured that the NL treatments occurred after flower initiation so that the effect of NL specifically on flower development could be assessed. The NLs were managed by pulling black cloth over the benches from 1800 HR to 0800 HR daily. After the SD treatments were provided, plants were randomly assigned to each of four NL treatments (11, 12, 13, or 14 h) until anthesis. The NL treatments were delivered by subdividing one bench into four sections ($1.83 \text{ m} \times 1.5 \text{ m} \times 0.9 \text{ m}$) separated by aluminized radiant barriers (Double Reflective Insulation; Reflectix Inc., Markleville, IN). Within each of the four bench sections, one white LED bulb (9W LED A19 Light Bulb, Utilitech) was hung above the plants to provide daylength-extension lighting ($1.3 \pm 0.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The LED bulbs were controlled with timers that turned the bulbs on at 1730 HR each day, and black cloth was pulled over the bench at the same time. Each evening, the LED bulb turned off at 1800, 1900, 2000, or 2100 HR in each of the four photoperiod sections to provide the 14-, 13-, 12-, or 11-h NL treatments, respectively. A black plastic sheet was hung from a wire between benches to prevent light pollution between treatments during the night. The plastic sheet was positioned between benches before 1800 HR and retracted after 0800 HR each day to minimize the blockage of sunlight.

Data collection. All plants were grown with a single, unpinched stem from which data were collected. The dates of first color, visible bud, and anthesis were recorded. Time to first color, visible bud, and anthesis were calculated as the number of days since the first SD. First color was identified when a green leaf had a distinctive blush of red pigmentation, indicating its development as a bract. A visible bud was identified when the primary cyathium was $\approx 2 \text{ mm}$ in diameter and clearly visible from an overhead view. Anthesis was identified on the primary cyathium when at least one stamen was releasing pollen. At the start of the SD treatment, a tag was hung from the most recently expanded mature leaf to count the number of nodes developing on the stem during the experiment. The following data were collected as individual plants reached anthesis: the number of nodes that developed during the experiment; the number of bracts forming on the stem below the terminal inflorescence (stem bract number); and color ratings for the stem bracts and the three primary bracts subtending the primary cyathium. Color ratings were assigned to leaves/bracts using a scale of 0 to 4 based on the surface area of the bract that developed red pigment: 0 = no red pigmentation; 1 = 1% to 25% red; 2 = 26% to 75% red; 3 = 76% to 99% red; and 4 = 100% red. Bracts rated as 4 are referred to as perfect

bracts. Color ratings were performed for the three primary bracts on each plant; however, these bracts were not distinguished from each other. Therefore, these data were averaged and presented as a single primary bract rating for each inflorescence.

Experimental design and analysis. The experiment was started on 24 Oct. 2019, and repeated on 9 Jan. 2020. The first replication included only the 17-d SD treatment and the four NL treatments. The second replication included the 10- and 17-d SD treatments and the four NL treatments. Two replications of the 10-d SD treatments were provided during the second experiment so that two complete replications of the 10- and 17-d treatments could be statistically analyzed. Data were analyzed using JMP Pro (version 14.0; SAS Institute Inc., Cary, NC). Analysis of variance tests were conducted to evaluate treatment effects, and the difference between treatment means were calculated using Fisher's least significant difference Student's *t* test ($P < 0.05$). For each experimental replication, each SD $\times$ NL treatment combination consisted of eight plants; however, the 10- and 17-d SD treatments that received the 14-h NL were not different because all of these plants were continuously grown under the 14-h NL for the duration of the experiment. Therefore, all data of plants with 10- and 17-d SD $\times$ 14-h NL treatments were pooled and referred to as the 14-h NL control.

Results and Discussion

Leaf number was unaffected by the experimental treatments (Table 1); e.g., the average number of leaves developing after the start of the SD treatments was 11.7 ± 0.8 . This suggests that flower initiation occurred during the 10-d SD treatment before the start of the NL treatments; therefore, the NL treatments applied during this study affected only flower development.

The interaction of SD $\times$ NL was significant for the time to the appearance of first color (Table 1). The 14-h NL control reached first color within 26.3 d, and no differences were observed with any treatment except 10-d SD $\times$ 11-h NL (Fig. 1A), which reached first color within 41.9 d. These results demonstrated that NLs of 12, 13, and 14 h are equivalent regarding the timing of first color when these NL treatments are applied after flower initiation; however, an 11-h NL will delay time to first color when 10-d SD treatment is provided, but not when 17-d SD treatment is provided. The additional 7 d under the 14-h NL with the 17-d SD $\times$ 11-h NL treatment likely allowed for the signal for anthocyanin synthesis and chlorophyll degradation to occur such that the timing of first color was unaffected even when plants were shifted to the 11-h NL.

The interaction of SD $\times$ NL was significant for time to visible bud (Table 1). The 14-h NL control reached visible bud within 38.8 d (Fig. 1B), whereas the 10-d SD $\times$ 11-h NL treatment was significantly delayed to 47.5 d. Time to visible bud was not different

Table 1. Statistical significance of the measured responses to the experimental treatments. Short days (SD), defined as a 14-h night length (NL), were provided for 10 or 17 d, then followed with four NL treatments (11, 12, 13, or 14 h). Leaf number was a measure of the number of nodes developed on the stem from the start of SD until the terminal cyathium developed. Time to first color and visible bud were measured as days from the start of SD to each response. Anthesis was measured as the percentage of plants reaching that stage of development. Stem bract number was measured as the number of bracts with a rating ≥ 1 , i.e., $\geq 1\%$ of bract surface contained red pigmentation at the time of anthesis.

Treatment	Leaf number	Time to first color	Time to visible bud	Anthesis (%)	Stem bract number
SD	NS	NS	***	NS	NS
NL	NS	***	***	***	***
SD $\times$ NL	NS	***	***	NS	***

NS, ***Nonsignificant or significant at $P < 0.05$, respectively.

for the 10-d SD $\times$ 12-h NL treatment compared with the 14-h NL control. Within the 17-d SD treatment, no differences were observed between the 14-h NL control and the 11-, 12-, and 13-h NL. These data demonstrate that an 11-h NL delays time to visible bud only when provided immediately after flower initiation, e.g., from 10 to 17 d after the start of SD. During this time, the involucre cup of

the primary cyathium forms and secondary cyathia meristems begin to differentiate in the axils of the primary bracts (Grueber and Wilkins, 1994). Similar to the development of first color, the early stages of flower development are sensitive to the photoperiod such that NL less than 12 h will slow the formation of these floral structures and delay the timing of visible bud.

The interaction of SD $\times$ NL was significant for the percentage of plants reaching anthesis (Table 1). Failure to reach anthesis occurred because of abortion of the primary cyathium within some treatments; therefore, time to anthesis data could not be collected and flowering percentages were more descriptive of the treatment responses. For the 14-h NL control, 97% of plants reached anthesis, which was not statistically different from the 12- or 13-h NL with either SD treatment (Fig. 1C); however, less than 40% of plants reached anthesis with the 11-h NL under both SD treatments. These data demonstrate that an 11-h NL inhibits normal flower development. Although the timing of visible bud was unaffected by the 11-h NL after 17-d SD treatment, this suboptimal NL clearly affects the capacity of the cyathia to develop from visible bud to anthesis. Langhans and Miller (1959) found similar results when poinsettia 'Barbara Ecke Supreme' was grown at NL from 12 to 16 h for 20 d, and then shifted to 11-h NL until anthesis. Anthesis failed to occur with these treatments.

The interaction of SD $\times$ NL had a significant effect on the number of stem bracts each treatment had at the time of anthesis (Table 1). The 14-h NL control had 8.0 stem bracts with a rating of 1 or more below the terminal inflorescence, which was not different from the 12- or 13-h NL in either SD treatment. However, both the 10- and 17-d SD $\times$ 11-h NL were different from the 14-h NL control and from each other; the 10-h $\times$ 11-h NL treatment had 4.1 stem bracts, whereas the 17-d $\times$ 11-h NL treatment had 5.6 stem bracts. These data demonstrate that an 11-h NL reduces the number of bracts that form during the development of the terminal inflorescence (i.e., the number of leaves that transition to bracts is affected by NL). The qualitative effect of the 11-h NL is not entirely captured by differences in the stem bract number because all bracts with a rating of 1 or more were included; therefore, the

bract ratings of the primary bracts and nine node positions below the terminal inflorescence were evaluated.

In the 10-d SD treatment, the primary bracts and stem bract positions 1 through 3 were rated as 4 (fully red) at the 12-, 13-, and 14-h NL (Fig. 2A). Bract ratings began to decrease below 4 at stem bract positions 4 through 6, but no differences in bract ratings were observed among the NL treatments at these positions. At stem bract positions 7 through 9, bract ratings for the 12-h NL treatment were significantly lower than those with the 13- and 14-h NL treatments. The 11-h NL was significantly different from the other NL treatments at all bract positions and never achieved a rating more than 3. In the 17-d SD treatment, the primary bracts and stem bract positions 1 through 3 were rated as 4 with the 12-, 13-, and 14-h NL (Fig. 2B). Bract ratings began to decrease from 4 at stem bract positions 4 and 5, but no differences in bract ratings were observed at these positions. At stem bract positions 6 and lower, bract ratings with the 12-h NL decreased much faster at each bract position relative to those with the 13- and 14-h NL. Bract ratings with the 11-h NL were typically higher after 17-d SD relative to 10-d SD, but the ratings associated with the 17-d SD $\times$ 11-h NL were significantly lower than those with the 12-, 13-, and 14-h NL at all bract positions except 8 and 9.

All plants subjected to the 11-h NL were commercially unacceptable by the end of the experiment, regardless of the SD treatment (Fig. 3). Poinsettia quality and market value are largely dictated by the abundance of color present in the bracts throughout the plant, especially in the primary bracts and the uppermost stem bracts. Time to first color was not delayed with the 17-d SD $\times$ 11-h NL treatment, but overall color development was clearly affected by this treatment. All plants treated with the 12-h NL were commercially acceptable but had lower quality overall compared with the 13- and 14-h NL because there was less red pigmentation in stem bract positions 6 and lower. Optimal color development occurred with the 13- and 14-h NL, and no visual differences were observed between plants grown under these two NL treatments.

Previous research (Alden and Faust, 2021) demonstrated large differences in the timing of anthesis when 'Prestige Red' was subjected to NLs of 11, 12, 13, or 14 h for the first 17 d of the experiment, followed by shifting all plants into the 14-h NL environment (e.g., time to anthesis occurred within 71, 67, 61, and 59 d, respectively, with the four NL treatments). However, the present study suggests that when the floral meristem has initiated, the effect of NL on the timing of first color, visible bud, and anthesis from 12 to 14 h is greatly reduced. For example, the number of days to anthesis were 66.1, 65.7, and 65.4 for the 12-, 13-, and 14-h NL treatments. The development of bract color has a longer NL optimum than the development of the cyathia because the plants subjected to the 13- and 14-h NL treatments had slightly superior quality relative to plants subjected to the 12-h NL

Fig. 1. Poinsettia 'Prestige Red' plants were initially placed under two short-day (SD) photoperiod treatments [14-h night lengths (NLs)] for 10 or 17 d before being moved to four NL treatments (11, 12, 13, or 14 h). Flowering responses measured included: (A) time to first color; (B) time to visible bud; and (C) percentage of plants reaching anthesis. The 10- and 17-d SD treatments at 14 h received this NL for the entire duration of the experiment; therefore, these data were pooled and are described as the 14-h NL control group. Error bars represent ± 1 SE.

Fig. 2. Poinsettia 'Prestige Red' plants were initially placed under two short-day (SD) photoperiod treatments [14-h night lengths (NLs)] for (A) 10 d or (B) 17 d to allow for flower initiation to occur before being moved to four NL treatments (11, 12, 13, or 14 h) so that NL effects on flower development could be recorded. Plants from the two SD treatments were followed by a 14-h NL for the remainder of the experiment. These plants received 14-h NL for the entire duration of the experiment; therefore, these data were pooled and are described as the 14-h NL control. Bract color ratings were recorded for the three primary bracts (PBs) and nine stem bracts (SBs 1–9) below the terminal inflorescence on reaching anthesis. The three PBs per shoot were not distinguished from each other; therefore, these data were pooled and presented as a single PB rating for each inflorescence. Bracts were rated using a scale of 0 to 4, where 0 = no red pigmentation, 1 = 1% to 25% red pigmentation, 2 = 26% to 75% red pigmentation, 3 = 76% to 99% red pigmentation, and 4 = 100% red pigmentation. Letters indicate significantly different bract ratings within each bract position across the two initial SD treatments using the least significant difference test ($\alpha = 0.05$). Error bars represent ± 1 SE.

treatments. Under natural NLs during the month of September at lat. 34.7°N, the NLs perceived by poinsettias are ≈ 10 h 44 min to 11 h 47 min (unpublished data). Data from this study along with that from the study by Alden and Faust (2021) suggest that these NLs are suboptimal for both flower initiation and development. NLs more than 12 h are not achieved until ≈ 6 Oct., which are acceptable for the development of the cyathia but are below the optimum for color development and flower initiation. Therefore, covering poinsettias with black cloth for 13-h or longer NLs is an effective method for accelerating

Fig. 3. Poinsettia 'Prestige Red' plants were placed under two short-day (SD) treatments [14-h night lengths (NLs) for 10 or 17 d] to allow flower initiation to occur before being moved to four NL treatments (11, 12, 13, or 14 h) so the effect on flower development could be observed. Photos were obtained 9 weeks after the beginning the initial 14-h NL period.

bract color development. Langhans and Larson (1959) and Langhans and Miller (1959) also found that poinsettias grown under 14- to 16-h NLs had superior quality in terms of bract color and salability relative to plants grown at NLs less than 14 h or natural-day photoperiods.

Our results and those reported by Alden and Faust (2021) demonstrate that modeling the flowering response of poinsettia requires NL to be considered separately for flower initiation and flower development. For both flowering processes, a 14-h NL results in rapid flower initiation, an optimal rate of flower development, and optimal bract color development. As NL decreases to less than 14 h, the rate of flower initiation is increasingly delayed, which causes slower overall crop response time. During flower development, NLs between 12 and 14 h have a minimal effect on crop response time, but a 12-h NL will negatively affect color development relative to 13- or 14-h NLs. NLs less than 12 h delay flower initiation and development and inhibit bract color development.

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